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DIVISION-CONTINUATION APPLICATION TRANSMITTAL FORM

Attorney Docket No.:

A-345-A

Anticipated Classification Of This Application:
Class

Subclass

Prior Application:
Examiner

Art Unit

To the Assistant Commissioner for Patents:

This is a request for filing a ☒ continuation ☐ divisional application, under 37 CFR 1.60, of pending prior application Serial No. 08/474,883 filed on June 7 19 95 ,
of MARY ANN PELLEYMOUNTER, RANDY IRA HECHT and MICHAEL BENJAMIN MANN
for OB PROTEIN COMPOSITIONS AND METHODS

1. ☒ Enclosed is a copy of the prior application, including the oath or declaration as originally filed. I hereby verify that the attached papers are a true copy of prior application Serial No. 08/474,833 as originally filed on June 7, 1995, and further that this Statement was made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

2. ☒ The filing fee is calculated below:

For	Number Filed		Number Extra		Rate	Fee
Total Claims	12	- 20 =	0	x	\$22.00 =	\$ 0.00
Independent Claims	4	- 3 =	1	x	\$80.00 =	80.00
Multiple Dependent Claims	0			+	\$260.00 =	0.00
Basic Fee					\$770.00 =	770.00
Total Filing Fee						\$ 850.00

3. ☒ The Commissioner is hereby authorized to charge any filing fees which may be required by the accompanying application, any additional fees which may be required during pendency of this application, or credit any over-payment to Deposit Account No. 01-0519 in the name of Amgen Inc. An original and one copy are enclosed.
4. ☐ A check in the amount of \$ _____ is enclosed.
5. ☐ Cancel in this application original claims _____ of the prior application before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)
6. ☒ Amend the specification by inserting before the first line the sentence: This application is a
☒ continuation, ☐ division, of application Serial No. 08/474,833, filed June 7, 1995 which is hereby incorporated by reference

EXPRESS MAIL CERTIFICATE

"Express Mail" mail labeling number: TB813684179

Date of Deposit: 8/27/97

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to Box Patent Application, Assistant Commissioner for Patents, Washington, D.C. 20231.

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Signature

7. ☐ Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this sheet is enclosed for filing in the prior application file. (May only be used if signed by person authorized by § 1.138 and before payment of base issue fee.)
- 7a. ☐ New formal drawings are enclosed.
8. ☐ Priority of application Serial No. _____ filed on _____ in _____ (country) is claimed under 35 U.S.C. 119.
- 8a. ☐ The certified copy has been filed in prior application Serial No. _____ filed _____
9. ☒ The prior application is assigned of record to AMGEN INC.
10. ☐ A preliminary amendment is enclosed.
11. ☒ Also enclosed is a Sequence Listing and Transmittal
12. ☒ The power of attorney in the prior application is to:
Ron K. Levy, Registration No.: 31,539; Steven M. Odre, Registration No.: 29,094; and
Karol M. Pessin, Registration No.: 34,899
- a. ☒ The power appears in the original papers in the prior application.
- b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.
- c. ☒ Address all future communications to
Karol M. Pessin
 at the address below.
- Signator: ☐ Assignee of complete interest
☒ Attorney or agent of record



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OB PROTEIN COMPOSITIONS AND METHODS

Field of the Invention

5 The present invention relates to OB protein compositions and methods for preparation and use thereof.

Background

10 Although the molecular basis for obesity is largely unknown, the identification of the "OB gene" and protein encoded by ("OB protein") has shed some light on mechanisms the body uses to regulate body fat deposition. Zhang et al., Nature 372: 425-432 (1994);
15 see also, the Correction at Nature 374: 479 (1995). The OB protein has been demonstrated to be active in vivo in both ob/ob mutant mice (mice obese due to a defect in the production of the OB gene product) as well as in normal, wild type mice. The biological activity
20 manifests itself in, among other things, weight loss. To date, however, optimum conditions for obtaining the rapid weight loss in normal animals has not been ascertained. In fact, some studies have shown that, when administered by injection, rather large dosages (10
25 mg of recombinant murine protein/kg body weight/day) are necessary for normal mice to lose 2.6% of their body weight (at the end of a 32 day period). While presently uncertain, one explanation for the necessity of such large dosages is that the optimum weight loss effects
30 are seen predominantly when the protein is in constant circulation, a condition that may not be efficiently achieved by injecting the protein.

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Date of Deposit June 7, 1995

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David F. Fellenz
Printed Name

David F. Fellenz 6/7/95
Signature

Summary of the Invention

The present invention stems from the observation that, as compared to administering OB protein by injection, administering OB protein by continuous pump infusion results in equivalent (or better) weight loss, in a shorter time, and with substantially lower dosages. The working example below demonstrates that a dose of 0.5 mg protein/kg body weight/day, administered via implantable osmotic pump, results in a weight loss of over 4% (as compared to baseline weight). This is in substantial contrast to other studies where similar, or less weight loss (at a comparable time point) was observed with intraperitoneal injection at the relatively high dosage of 10 mg of protein/kg body weight/day.

Thus, one aspect of the present invention is a method of treating excess weight by administering OB protein in a form for constant supply, at a dosage of less than or equal to about 1 mg protein/kg body weight/day. The dosage of less than or equal to about 1 mg protein/kg/day refers to dosages sufficient to result in observable weight loss. This is apparent from the present studies where a dosage of 0.5 mg/kg/day was sufficient to result in observable weight loss when continuously administered. In studies where injection had been the mode of administration, far higher dosages were required for weight loss. At injection dosages of 0.1 and 1 mg/kg/day, substantially no weight loss was observed in wild type (normal) mice. For example, in one study, at a comparable time point (6th day), there was a .2% loss at the 1 mg/kg dose (data not shown). Minimal weight loss was observed at the relatively high 10 mg/kg/day dose. (1.9% weight loss at day 6, data not shown). Thus, the present invention provides for dosages of 1 mg/kg/day or less when administered so that the supply of protein is continuous.

Connected with the present studies are the compositions and methods used for production of recombinant murine and human OB protein. The first example below discloses the preparation of recombinant murine protein, and the second example below discloses the preparation of recombinant human protein.

Additional aspects of the present invention, therefore, include the below compositions and methods for preparing recombinant murine methionyl OB protein and recombinant human methionyl OB protein, including DNA sequences, vectors, host cells, methods of fermentation, and methods of purification.

Detailed Description

The present invention stems from the observation that continuous administration of OB protein results in the need for much lower dosages for weight loss than those dosages required by acute daily injection. As set forth above, a dosage of 1 mg protein/kg body weight/day or less, continuously administered, resulted in rapid weight loss. When the underivatized protein was administered by acute injection at the 1 mg/kg/day dose, almost no weight loss in wild type (normal) mice occurred.

The OB protein may be selected from the recombinant murine and human methionyl proteins set forth below (SEQ. ID Nos. 2 and 4) or those lacking a glutaminy residue at position 28. (See Zhang et al, Nature, supra, at page 428.) The recombinant human OB gene product is, as a mature protein, 146 amino acids; some of the DNAs obtained were observed to encode a protein lacking a glutamine residue at position 28. Zhang et al., Nature 372 at 428. The murine protein is substantially homologous to the human protein, particularly as a mature protein, and, further, particularly at the N-terminus. One may prepare an

analog of the recombinant human protein by altering (such as substituting amino acid residues), in the recombinant human sequence, the amino acids which diverge from the murine sequence. Because the recombinant human protein has biological activity in mice, such analog would likely be active. Proteins lacking an N-terminal methionyl residue, such as those produced by eukaryotic expression, are also available for use.

10 In addition, although the present working example involved continuous administration via implantable pump, it is contemplated that other modes of continuous administration may be practiced. For example, chemical derivatization may result in sustained release forms of the protein which have the effect of continuous presence in the blood stream, in predictable amounts. Thus, one may derivatize the above proteins to effectuate such continuous administration. The dosage of 1 mg protein/kg body weight/day or less herein refers to the mass of protein, exclusive of other chemical moieties used to derivatize the protein.

Generally, the present protein (herein the term "protein" is used to include "peptide", unless otherwise indicated) may be derivatized by the attachment of one or more chemical moieties to the protein moiety. The chemically modified derivatives may be further formulated for intraarterial, intraperitoneal, intramuscular subcutaneous, intravenous, oral, nasal, pulmonary, topical or other routes of administration. Chemical modification of biologically active proteins has been found to provide additional advantages under certain circumstances, such as increasing the stability and circulation time of the therapeutic protein and decreasing immunogenicity. See U.S. Patent No. 4,179,337, Davis et al., issued December 18, 1979. For a review, see Abuchowski et al., in

Enzymes as Drugs. (J.S. Holcerberg and J. Roberts, eds. pp. 367-383 (1981)). A review article describing protein modification and fusion proteins is Francis, Focus on Growth Factors 3: 4-10 (May 1992) (published by Mediscript, Mountview Court, Friern Barnet Lane, London N20, OLD, UK). For the present continuous administration, it is preferred that the chemical modification allow for an increase in circulation time of the protein, so that a dosage of about 1 mg protein (exclusive of chemical moiety)/kg body weight of a mammal/day or less will result in weight loss of a mammal. The present continuous administration will provide for weight loss of approximately 5% of body mass in a period of 7 or fewer days.

The chemical moieties suitable for derivatization may be selected from among water soluble polymers. The polymer selected should be water soluble so that the protein to which it is attached does not precipitate in an aqueous environment, such as a physiological environment. Preferably, for therapeutic use of the end-product preparation, the polymer will be pharmaceutically acceptable. One skilled in the art will be able to select the desired polymer based on such considerations as whether the polymer/protein conjugate will be used therapeutically, and if so, the desired dosage, circulation time, resistance to proteolysis, and other considerations. For the present proteins and peptides, the effectiveness of the derivatization may be ascertained by administering the derivative, in the desired form (i.e., by osmotic pump, or, more preferably, by injection or infusion, or, further formulated for oral, pulmonary or nasal delivery, for example), and measuring weight loss.

The water soluble polymer may be selected from the group consisting of, for example, polyethylene glycol, copolymers of ethylene glycol/propylene glycol,

by factors such as the desired degree of derivatization (e.g., mono, di-, tri-, etc.), the molecular weight of the polymer selected, whether the polymer is branched or unbranched, and the reaction conditions.

5 The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art. E.g., EP 0 401 384 herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20: 1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residue. Those having a free carboxyl group may include aspartic acid residues, glutamic acid residues, and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecule(s). Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group. Attachment at residues important for receptor binding should be avoided if receptor binding is desired.

30 One may specifically desire N-terminally chemically modified protein. Using polyethylene glycol as an illustration of the present compositions, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (or peptide) molecules in the reaction mix, the type of pegylation

reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective N-terminal chemical modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved. For example, one may selectively N-terminally pegylate the protein by performing the reaction at a pH which allows one to take advantage of the pK_a differences between the ϵ -amino group of the lysine residues and that of the α -amino group of the N-terminal residue of the protein. By such selective derivatization, attachment of a water soluble polymer to a protein is controlled: the conjugation with the polymer takes place predominantly at the N-terminus of the protein and no significant modification of other reactive groups, such as the lysine side chain amino groups, occurs. Using reductive alkylation, the water soluble polymer may be of the type described above, and should have a single reactive aldehyde for coupling to the protein. Polyethylene glycol propionaldehyde, containing a single reactive aldehyde, may be used.

In yet another aspect of the present invention, provided are methods of using pharmaceutical compositions of the proteins and derivatives. Such pharmaceutical compositions may be for administration for injection, or for oral, pulmonary, nasal or other forms of administration which allow for the desired

circulating dose of about 1 mg protein/kg body weight/day or less. In general, comprehended by the invention are pharmaceutical compositions comprising effective amounts of protein or derivative products of the invention together with pharmaceutically acceptable diluents, preservatives, solubilizers, emulsifiers, adjuvants and/or carriers. Such compositions include diluents of various buffer content (e.g., Tris-HCl, acetate, phosphate), pH and ionic strength; additives such as detergents and solubilizing agents (e.g., Tween 80, Polysorbate 80), anti-oxidants (e.g., ascorbic acid, sodium metabisulfite), preservatives (e.g., Thimersol, benzyl alcohol) and bulking substances (e.g., lactose, mannitol); incorporation of the material into particulate preparations of polymeric compounds such as polylactic acid, polyglycolic acid, etc. or into liposomes. Hylauronic acid may also be used, and this may have the effect of promoting sustained duration in the circulation. Such compositions may influence the physical state, stability, rate of in vivo release, and rate of in vivo clearance of the present proteins and derivatives. See, e.g., Remington's Pharmaceutical Sciences, 18th Ed. (1990, Mack Publishing Co., Easton, PA 18042) pages 1435-1712 which are herein incorporated by reference. The compositions may be prepared in liquid form, or may be in dried powder, such as lyophilized form. The effective amounts are those herein described.

The OB proteins and derivatives described are useful for modulation of the rate or quantity of fat cell deposition in a mammal. This is thought to be accomplished, in part, by a reduction in appetite, i.e., a reduction in food intake. Thus, one observable result is weight loss, or, put another way, a method of treating excess weight (via weight loss). Thus, the present compositions are useful for the manufacture of a medicament for treating excess weight in a mammal.

Another aspect is a method for reducing appetite. Either of these aspects, modulation of fat deposition or modulation of appetite, are particularly important treatments for humans (or other mammals) who desire to
5 lose weight.

One skilled in the art will be able to ascertain other effective dosages by administration and observing weight loss. Here, the dosage of 1 mg protein/kg body weight/day or less was seen to be
10 particularly effective, when administered on a continuous basis. More particularly, the dosage of 0.5 mg/kg body weight/day was seen to be particularly effective on normal mice. Excess weight refers to body mass for which removal is desired. It is contemplated
15 that the present compositions and methods will be used to treat cases where removal of such excess weight (as a result of the present invention) will benefit other health concerns, such as diabetes, high blood pressure or cardiac problems, high cholesterol levels, low
20 locomotion levels and other manifestations of excess weight. As such, the present compositions and methods may be used in conjunction with other medicaments, such as those useful for the treatment of diabetes (e.g., insulin, and possibly amylin), cholesterol and blood
25 pressure lowering medicaments, and locomotion increasing medicaments (e.g., amphetamines). Such administration may be simultaneous or may be in serriatim.

In addition, the present compositions and methods may be used in conjunction with surgical
30 procedures, such as cosmetic surgeries designed to alter the overall appearance of a body (e.g., liposuction or laser surgeries designed to reduce body mass). The health benefits of cardiac surgeries may be increased with concomitant use of the present compositions and
35 methods.

Therefore, the present invention encompasses a method of treating excess weight in a mammal by continuous administration of 1 mg protein/kg body weight/day or less of an OB protein selected from the group consisting of:

(a) recombinant methionyl murine OB protein (SEQ. ID. No. 2);

(b) recombinant methionyl human OB protein (SEQ ID No. 1);

(c) the protein of (a) or (b) lacking the methionyl residue at position -1;

(d) the protein of (a), (b) or (c) lacking a glutamine at position 28; and

(e) a chemically modified derivative of (a), (b), (c) or (d), wherein the chemical modification allows for an increase in circulation time.

Preferably, the composition of subpart (e) is a pegylated derivative, and, more preferably, an N-terminally pegylated derivative.

The derivative of subpart (e) allows for continuous administration of the protein by increasing the circulation time of the (unmodified) protein. The present invention also encompasses a method of treating excess weight where the method of continuous administration is by implantable pump, such as an osmotic pump.

In other aspects, the present invention relates to recombinant murine and recombinant human OB DNAs and proteins, such as those of SEQ. ID NOs. 1, 2, 3, and 4, below. The recombinant proteins below are bacterially expressed, and contain N-terminal methionyl residues. Vectors and host cells useful for producing such proteins are also provided. The vectors include pCFM1656 containing SEQ ID No. 1 or 3, and host cells containing such vectors.

Methods for preparation of the recombinant proteins are also provided, including methods for fermentation and methods for purification.

In particular, the use of sarcosine for
5 refolding of OB protein in solution, obtained from
bacterial inclusion bodies, provided for extremely
efficient refolding. When proteins are expressed in
bacteria, they may not be in the proper three-
dimensional configuration, or, as referred to herein,
10 properly refolded. The three dimensional configuration
may be critical for biological activity, and storage
stability. Although Sarckosyl has been used in processes
for purification of another protein (G-CSF, e.g., WO
89/10932), surprisingly, the use of sarcosine for the OB
15 protein has resulted in a refolding efficiency of over
95%. Contemplated herein is the use of N-
lauroylsarcosine in a range of 0.5% - 2.0 % weight per
volume of OB protein in solution (obtained from
inclusion bodies). With the use of 1% sodium sarcosine,
20 the refolding efficiency, as determined by SDS PAGE and
reverse phase HPLC, was 95% or greater. While one
skilled in the art will recognize that other
compositions may be used for refolding, the use of
N-lauroyl sarcosine, as illustrated in the examples
25 below, is particularly advantageous for providing
extremely efficient refolding. The removal of sarcosine
was accomplished using Dowex®.

Therefore, the present invention also includes
a method of refolding partially purified OB protein in a
30 solution obtained from inclusion bodies, said partially
purified OB protein selected from the group consisting
of:

- (a) recombinant methionyl murine OB protein
(SEQ. ID. No. 2);
- 35 (b) recombinant methionyl human OB protein
(SEQ ID No. 1);

(c) the protein of (a) or (b) lacking the methionyl residue at position -1;

wherein said refolding is accomplished using sarcosine.

5 The present invention also includes methods of wherein said N-lauroyl sarcosine is used at a concentration of 0.5% - 2.0% weight per volume of solution, and, more particularly, the use of 1% N-lauroyl sarcosine. An oxidizing agent, such as copper
10 sulfate, is also used in the refolding process.

The following examples are offered to more fully illustrate the invention, but are not to be construed as limiting the scope thereof.

15 EXAMPLE 1: Use of Murine OB Protein in a Continuous Pump Infusion System

This example demonstrates that continuous infusion of OB protein results in weight loss in normal mice. Normal (non-obese) mice were administered murine
20 OB protein via osmotic pump infusion. A dosage of 0.5 mg protein/kg body weight/day resulted in a 4.62% (+/- 1.34%) loss from baseline weight by the 6th day of infusion.

25 MATERIALS AND METHODS

Animals: Wild type (+/+) C57B16 mice were used for this experiment. The age of the mice at the initial time point was 8 weeks, and the animals were weight
30 stabilized. 10 mice were used for each cohort (vehicle vs. protein).

Animal Handling.

Feeding and weight measurement. Mice were given
35 ground rodent chow (PMI Feeds, Inc.) in powdered food feeders (Allentown Caging and Equipment) which allowed a

more accurate and sensitive measurement than use of regular block chow. Weight was measured at the same time each day (2:00 p.m.), for a period of 6 days. Body weight on the day prior to the infusion was defined as
5 baseline weight. The mice used weighed 18-22 grams.

Housing. Mice were single-housed, and maintained under humane conditions.

10 Administration of Protein or Vehicle. Protein (as described below) or vehicle (phosphate buffered saline, pH 7.4) were administered by osmotic pump infusion. Alzet osmotic minipumps (Alza, Palo Alto, CA, model no. 1007D) were surgically placed in each mice in a
15 subcutaneous pocket in the subscapular area . The pumps were calibrated to administer 0.5 μ l protein in solution per hour for a dosage of 0.5 mg protein/kg body weight/day.

20 Controls: Control animals were those who had a Alzet osmotic minipump infusing phosphate buffered saline (pH 7.4).

Protein: Recombinant murine OB protein was used for
25 the present experiments, generally at a concentration of about 0.9 mg/ml phosphate buffered saline, pH 7.4. The amino acid sequence (and DNA sequence) used was the following:

08920508-082797

Region	Area	Population	Area	Population
1	100	100	100	100
2	100	100	100	100
3	100	100	100	100
4	100	100	100	100
5	100	100	100	100
6	100	100	100	100
7	100	100	100	100
8	100	100	100	100
9	100	100	100	100
10	100	100	100	100
11	100	100	100	100
12	100	100	100	100
13	100	100	100	100
14	100	100	100	100
15	100	100	100	100
16	100	100	100	100
17	100	100	100	100
18	100	100	100	100
19	100	100	100	100
20	100	100	100	100
21	100	100	100	100
22	100	100	100	100
23	100	100	100	100
24	100	100	100	100
25	100	100	100	100
26	100	100	100	100
27	100	100	100	100
28	100	100	100	100
29	100	100	100	100
30	100	100	100	100
31	100	100	100	100
32	100	100	100	100
33	100	100	100	100
34	100	100	100	100
35	100	100	100	100
36	100	100	100	100
37	100	100	100	100
38	100	100	100	100
39	100	100	100	100
40	100	100	100	100
41	100	100	100	100
42	100	100	100	100
43	100	100	100	100
44	100	100	100	100
45	100	100	100	100
46	100	100	100	100
47	100	100	100	100
48	100	100	100	100
49	100	100	100	100
50	100	100	100	100
51	100	100	100	100
52	100	100	100	100
53	100	100	100	100
54	100	100	100	100
55	100	100	100	100
56	100	100	100	100
57	100	100	100	100
58	100	100	100	100
59	100	100	100	100
60	100	100	100	100
61	100	100	100	100
62	100	100	100	100
63	100	100	100	100
64	100	100	100	100
65	100	100	100	100
66	100	100	100	100
67	100	100	100	100
68	100	100	100	100
69	100	100	100	100
70	100	100	100	100
71	100	100	100	100
72	100	100	100	100
73	100	100	100	100
74	100	100	100	100
75	100	100	100	100
76	100	100	100	100
77	100	100	100	100
78	100	100	100	100
79	100	100	100	100
80	100			

5	9	TCTAGATTTGAGTTTAACTTTTAACTTTTGAAGGAGGAATAACATATGGTACCGATCCAGAAAGT	68
		AGATCTAAACTCAAAATTGAAAATCTTCCCTCCTTATTGTATACCATGGCTAGGTCTTTCA	
		M V P I Q K V -	
10	69	TCAGGACGACACCAAAACCTTAATTAAAACGATCGTTACGCGTATCAACGACATCAGTCA	128
		AGTCCTGCTGTGGTTTTGAATTAATTTTGCTAGCAATGCGCATAGTTGCTGTAGTCAGT	
		Q D D T K T L I K T I V T R I N D I S H -	
15	129	CACCCAGTCGGTCTCCGCTAAACAGCGTGTTACCGGTCTGGACTTCATCCCGGTCTGCA	188
		GTGGGTCAGCCAGAGGCGATTGTGCGACAATGGCCAGACCTGAAGTAGGGCCCAGACGT	
		T Q S V S A K Q R V T G L D F I P G L H -	
20	189	CCCGATCCTAAGCTTGTCCAAAATGGACCAGACCCTGGCTGTATACCAGCAGGTGTTAAC	248
		GGGCTAGGATTGGAACAGGTTTACCTGGTCTGGGACCGACATATGGTCGTCCACAATTG	
		P I L S L S K M D Q T L A V Y Q Q V L T -	
25	249	CTCCCTGCCGTCCCAGAACGTTCTTCAGATCGCTAACGACCTCGAGAACCTTCGCGACCT	308
		GAGGGACGGCAGGGTCTTGCAAGAAGTCTAGCGATTGCTGGAGCTCTTGGAAGCGCTGGA	
		S L P S Q N V L Q I A N D L E N L R D L -	
30	309	GCTGCACCTGCTGGCATTCTCCAAATCCTGCTCCCTGCCGCAGACCTCAGGTCTTCAGAA	368
		CGACGTGGACGACCGTAAGAGGTTTAGGACGAGGGACGGCGTCTGGAGTCCAGAAGTCTT	
		L H L L A F S K S C S L P Q T S G L Q K -	
35	369	ACCGGAATCCCCTGGACGGGGTCCCTGGAAGCATCCCTGTACAGCACCGAAGTTGTTGCTCT	428
		TGGCCTTAGGGACCTGCCCCAGGACCTTCGTAGGGACATGTCGTGGCTTCAACAACGAGA	
		P E S L D G V L E A S L Y S T E V V A L -	
40	429	GTCCCGTCTGCAGGGTTCCCTTCAGGACATCCTTCAGCAGCTGGACGTTTCTCCGGAATG	488
		CAGGGCAGACGTCCCAAGGGAAGTCCTGTAGGAAGTCGTCGACCTGCAAAGAGGCCTTAC	
		S R L Q G S L Q D I L Q Q L D V S P E C -	
45	489	TTAATGGATCC	
		AATTACCTAGG	

Herein, the first amino acid of the amino acid sequence for recombinant protein is referred to as +1, and is valine, and the amino acid at position -1 is methionine. The C-terminal amino acid is number 146 (cysteine).

The cloning of the murine OB DNA for expression in E. coli was done as follows. The DNA sequence was deduced from the published peptide sequence that appeared in Zhang et al., Nature 372:425-432 (1994). It was reverse translated using E. coli optimal codons. The terminal cloning sites were XbaI to BamHI. A ribosomal binding enhancer and a strong ribosomal binding site were included in front of the coding region. The duplex DNA sequence was synthesized using standard techniques. Correct clones were confirmed by demonstrating expression of the recombinant protein and presence of the correct OB DNA sequence in the resident plasmid.

Expression Vector and Host Strain

The plasmid expression vector used was pCFM1656, ATCC Accession No. 69576. The above DNA was ligated into the expression vector pCFM1656 which had been linearized with XbaI and BamHI and transformed into the E. coli host strain, FM5. E. coli FM5 cells were derived at Amgen Inc., Thousand Oaks, CA from E. coli K-12 strain (Bachmann, et al., Bacteriol. Rev. 40: 116-167 (1976)) and contain the integrated lambda phage repressor gene, cI₈₅₇ (Sussman et al., C.R. Acad. Sci. 254: 1517-1579 (1962)). Vector production, cell transformation, and colony selection were performed by standard methods. E.g., Sambrook, et al., Molecular Cloning: A Laboratory Manual, 2d Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Host cells were grown in LB media.

Fermentation Process A three-phase fermentation protocol was used known as a fed-batch process. Media compositions are set forth below.

5 Batch: A nitrogen and phosphate source were
sterilized (by raising to 122 °C for 35 minutes, 18-20
psi) in the fermentation vessel (Biolafitte, 12 liter
capacity). Upon cooling, carbon, magnesium, vitamin,
and trace metal sources were added aseptically. An
10 overnight culture of the above recombinant murine
protein-producing bacteria (16 hours or more) of 500 mL
(grown in LB broth) was added to the fermentor.

 Feed I: Upon reaching between 4.0-6.0 OD₆₀₀,
15 cultures were fed with Feed I. The glucose was fed at a
limiting rate in order to control the growth rate (μ) .
An automated system (called the Distributive Control
System) was instructed to control the growth rate to
0.15 generations per hour.

20 Feed II: When the OD₆₀₀ had reached 30,
culture temperature was slowly increased to 42°C and the
feed was changed to Feed II, below. The fermentation was
then allowed to continue for 10 hours with sampling
25 every 2 hours. After 10 hours, the contents of the
fermentor was chilled to below 20°C and harvested by
centrifugation.

Media Composition:

	Batch:	10 g/L	Yeast extract
		5.25 g/L	(NH ₄) ₂ SO ₄
		3.5 g/L	K ₂ HPO ₄
5		4.0 g/L	KH ₂ PO ₄
		5.0 g/L	Glucose
		1.0 g/L	MgSO ₄ ·7H ₂ O
		2.0 mL/L	Vitamin Solution
		2.0 mL/L	Trace Metal Solution
10		1.0 mL/L	P2000 Antifoam
	Feed I:	50 g/L	Bacto-tryptone
		50 g/L	Yeast extract
		450 g/L	Glucose
		8.75 g/L	MgSO ₄ ·7H ₂ O
15		10 mL/L	Vitamin Solution
		10 mL/L	Trace Metal Solution
	Feed II:	200 g/L	Bacto-tryptone
		100 g/L	Yeast extract
		110 g/L	Glucose

20

Vitamin Solution (Batch and Feed I):

0.5 g Biotin, 0.4 g Folic acid, and 4.2 g riboflavin, were dissolved in 450 mls H₂O and 3 mls 10 N NaOH, and brought to 500 mls in H₂O. 14 g pyridoxine-HCl and 61 g niacin were dissolved 150 ml H₂O and 50 ml 10 N NaOH, and brought to 250 ml in H₂O. 54 g pantothenic acid was dissolved in 200 ml H₂O, and brought to 250 ml. The three solutions were combined and brought to 10 liters total volume.

08020509 08020509

Purification was accomplished by the following steps (unless otherwise noted, the following steps were performed at 4°C):

2. Inclusion body wash #1. The supernatant from above was removed, and the pellet was resuspended with 5 times volume of 7 mM EDTA, pH 7.0, and homogenized. This mixture was centrifuged as in step 1.

3. Inclusion body wash #2. The supernatant from above was removed, and the pellet was resuspended in ten times volume of 20 mM tris, pH 8.5, 10 mM DTT, and 1% deoxycholate, and homogenized. This mixture was centrifuged as in step 1.

4. Inclusion body wash #3. The supernatant from above was removed and the pellet was resuspended in ten times volume of distilled water, and homogenized. This mixture was centrifuged as in step 1.

5. Refolding. The pellet was refolded with 15 volumes of 10 mM HEPES, pH 8.5, 1% sodium sarcosine (N-lauroyl sarcosine), at room temperature. After 60 minutes, the solution is made to be 60 μ M copper

5 sulfate, and then stirred overnight.

6. Removal of sarcosine. The refolding mixture was diluted with 5 volumes of 10 mM tris buffer, pH 7.5, and centrifuged as in step 1. The supernatant was collected, and mixed with agitation for one hour with
10 Dowex® 1-X4 resin (Dow Chemical Co., Midland MI), 20-50 mesh, chloride form, at 0.066% total volume of diluted refolding mix. See WO 89/10932 at page 26 for more information on Dowex®. This mixture was poured into a column and the eluant was collected. Removal of
15 sarcosine was ascertained by reverse phase HPLC.

7. Acid precipitation. The eluant from the previous step was collected, and pH adjusted to pH 5.5, and incubated for 30 minutes at room temperature. This mixture was centrifuged as in step 1.

20 8. Cation exchange chromatography. The pH of the supernatant from the previous step was adjusted to pH 4.2, and loaded on CM Sepharose Fast Flow (at 7% volume). 20 column volumes of salt gradient were done at 20 mM NaOAC, pH 4.2, 0 M to 1.0 M NaCl.

25 9. Hydrophobic interaction chromatography. The CM Sepharose pool of peak fractions (ascertained from ultraviolet absorbance) from the above step was made to be 0.2 M ammonium sulfate. A 20 column volume reverse salt gradient was done at 5 mM NaOAC, pH 4.2, with .4 M
30 to 0 M ammonium sulfate. This material was concentrated and diafiltered into PBS.

Results

Presented below are the percent (%) differences from baseline weight in C57Bl6J mice (8 weeks old) :

5

Table 1: Weight Loss Upon Continuous Infusion

<u>Time (days)</u>	<u>Vehicle (PBS)</u>	<u>Recombinant OB protein</u>
Days 1-2	3.24 +/- 1.13	1.68 +/- 1.4
Days 3-4	4.3 +/- .97	-2.12 +/- .79
Days 5-6	4.64 +/- .96	-4.62 +/- 1.3

As can be seen, at the end of a 6 day continuous infusion regime, animals receiving the OB protein lost over 4% of their body weight, as compared to baseline. This is a substantially more rapid weight loss than has been observed with intraperitoneal (i.p.) injection. Weight loss at the end of a 32-day injection period, in wild type (normal) mice, with daily i.p. injections of recombinant murine OB protein at a 10 mg/kg dose was 2.6%, and had not been more than 4% at any time during the dosing schedule (data not shown). The present data indicate that with continuous infusion, a 20-fold lower dosage (0.5 mg/kg vs. 10 mg/kg) achieves more weight loss in a shorter time period.

The results seen here are statistically significant, e.g., -4.62% with $p < .0001$.

EXAMPLE 2: Dose Response Studies

An additional study demonstrated that there was a dose response to continuous administration of OB protein. In this study, non-obese, CD-1 mice, weighing 35-40 g were administered recombinant murine OB protein using methods similar to the above example. The results are set forth in Table 2, below, (with % body weight lost as compared to baseline, measured as above):

Table 2: Dose Response With Continuous Administration

Dose	Time	% Reduction in body weight
0.03 mg/kg/day	Day 2	3.5
1 mg/kg/day	Day 2	7.5
1 mg/kg/day	Day 4	14

As can be seen, increasing the dose from 0.03 mg/kg/day to 1 mg/kg/day increased the weight lost from 3.5% to 7.5%. It is also noteworthy that at day 4, the 1 mg/kg/day dosage resulted in a 14% reduction in body weight.

EXAMPLE 3: Cloning and Expression of a Recombinant Human Methionyl OB Protein

This example provides compositions and methods for preparation of a recombinant human version of the OB protein.

The recombinant human version of the OB DNA was constructed from the murine OB DNA, as in Example 1, above, by replacing the region between the MluI and BamHI sites with duplex DNA (made from synthetic oligonucleotides) in which 20 codon substitutions had been designed. The MluI site is shown under the solid line in the sequence below. This DNA was put into the pCFM1656

Table 1. Demographic characteristics of the study population	
Age (years)	65.0 ± 10.0
Gender	
Male	50 (50.0%)
Female	50 (50.0%)
Education (years)	12.0 ± 2.0
Marital status	
Married	40 (80.0%)
Single	10 (20.0%)
Occupation	
Retired	30 (60.0%)
Unemployed	20 (40.0%)
Income (USD/month)	1,200 ± 300
Health status	
Good	30 (60.0%)
Fair	20 (40.0%)
Poor	10 (20.0%)
Comorbidities	
Hypertension	20 (40.0%)
Diabetes	10 (20.0%)
Cholesterol	15 (30.0%)
Arthritis	10 (20.0%)
Other	5 (10.0%)
Medication	
Yes	30 (60.0%)
No	20 (40.0%)
Smoking status	
Smoker	10 (20.0%)
Non-smoker	40 (80.0%)
Alcohol consumption	
Yes	10 (20.0%)
No	40 (80.0%)
Family size	3.0 ± 1.0
Living alone	10 (20.0%)
Living with family	40 (80.0%)
Health insurance	
Yes	30 (60.0%)
No	20 (40.0%)
Access to healthcare	
Yes	30 (60.0%)
No	20 (40.0%)
Healthcare utilization	
Yes	30 (60.0%)
No	20 (40.0%)
Healthcare satisfaction	
Satisfied	30 (60.0%)
Dissatisfied	20 (40.0%)
Healthcare access barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare cost barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare quality barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
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No	40 (80.0%)
Healthcare provider barriers	
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Healthcare facility barriers	
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No	40 (80.0%)
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No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare facility barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare information barriers	
Yes	10 (20.0%)
No	40 (80.0%)
Healthcare provider barriers	
Yes	10 (20.0%)
No	

Recombinant human met OB (Double Stranded) DNA and amino acid sequence (Seq. ID. Nos. 3 and 4)

```

5      CATATGGTACCGATCCAGAAAGTTCAGGACGACACCAAAACCTTAATTAAAACGATCGTT
      1  -----+-----+-----+-----+-----+-----+ 60
      GTATACCATGGCTAGGTCTTTCAAGTCCTGCTGTGGTTTTGGAATTAATTTTGCTAGCAA
          M V P I Q K V Q D D T K T L I K T I V -

10     ACGCGTATCAACGACATCAGTCACACCCAGTCGGTGAGCTCTAAACAGCGTGTTACAGGC
      61  -----+-----+-----+-----+-----+-----+ 120
      TGCGCATAGTTGCTGTAGTCAGTGTGGGTCAGCCACTCGAGATTTGTGCGACAATGTCCG
          T R I N D I S H T Q S V S S K Q R V T G -

15     CTGGACTTCATCCCGGGTCTGCACCCGATCCTGACCTTGTC AAAATGGACCAGACCCCTG
      121 -----+-----+-----+-----+-----+-----+ 180
      GACCTGAAGTAGGGCCAGACGTGGGCTAGGACTGGAACAGGTTTTACCTGGTCTGGGAC
          L D F I P G L H P I L T L S K M D Q T L -

20     GCTGTATACCAGCAGATCTTAACCTCCATGCCGTCCCGTAACGTTCTTCAGATCTCTAAC
      181 -----+-----+-----+-----+-----+-----+ 240
      CGACATATGGTCGTCTAGAATTGGAGGTACGGCAGGGCATTGCAAGAAGTCTAGAGATTG
          A V Y Q Q I L T S M P S R N V L Q I S N -

30     GACCTCGAGAACCTTCGCGACCTGCTGCACGTGCTGGCATTCTCCAAATCCTGCCACCTG
      241 -----+-----+-----+-----+-----+-----+ 300
      CTGGAGCTCTTGGAAGCGCTGGACGACGTGCACGACCGTAAGAGGTTTAGGACGGTGGAC
          D L E N L R D L L H V L A F S K S C H L -

35     CCATGGGCTTCAGGTCTTGAGACTCTGGACTCTCTGGGCGGGGTCCTGGAAGCATCCGGT
      301 -----+-----+-----+-----+-----+-----+ 360
      GGTACCCGAAGTCCAGAACTCTGAGACCTGAGAGACCCGCCCCAGGACCTTCGTAGGCCA
          P W A S G L E T L D S L G G V L E A S G -

40     TACAGCACCGAAGTTGTTGCTCTGTCCCGTCTGCAGGGTTCCCTTCAGGACATGCTTTGG
      361 -----+-----+-----+-----+-----+-----+ 420
      ATGTCGTGGCTTCAACAACGAGACAGGGCAGACGTCCCAAGGGAAGTCCTGTACGAAACC
          Y S T E V V A L S R L Q G S L Q D M L W -

50     CAGCTGGACCTGTCTCCGGTTGTTAATGGATCC
      421 -----+-----+-----+-----+ 454
      GTCGACCTGGACAGAGGCCCAACAATTACCTAGG
          Q L D L S P G C *
55

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66260"5972630

Fermentation: Fermentation of the above host cells to produce recombinant human OB protein was accomplished using the conditions and compositions as described above for recombinant murine material. The results were analyzed for yield (grams ob DNA product/liter of fermentation broth), prior to purification of the recombinant human OB material. (Minor amounts of bacterial protein were present.) Bacterial expression was also calculated.

Table 3: Analysis of Recombinant Human OB Protein Expression

Timepoint	OD (@600 nm)	Yield (g/L)	Expression (mg/OD·L)
Ind. + 2 hours.	47	1.91	41
Ind. + 4 hours.	79	9.48	120
Ind. + 6 hours.	95	13.01	137
Ind. + 8 hours.	94	13.24	141
Ind. + 10 hours.	98	14.65	149

abbreviations: Ind. + __ hours means the hours after induction of protein expression, as described in Example I for the recombinant murine material using pCFM1656
 OD: optical density, as measured by spectrophotometer
 milligrams per OD unit per liter
 mg/OD·L: expression in terms of milligrams of protein per OD unit per liter.

g/L: grams protein/liter fermentation broth

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10

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CLAIMS

1. A method of treating excess weight in a
5 mammal by continuous administration of 1 mg protein/kg
body weight/day or less of an OB protein selected from
the group consisting of:
- (a) recombinant methionyl murine OB protein
(SEQ. ID. No. 2);
 - 10 (b) recombinant methionyl human OB protein
(SEQ ID No. 1);
 - (c) the protein of (a) or (b) lacking the
methionyl residue at position -1;
 - (d) the protein of (a), (b) or (c) lacking a
15 glutamine at position 28; and
 - (e) a chemically modified derivative of (a),
(b), (c) or (d).
2. A method of claim 1 wherein the chemically
20 modified derivative is a pegylated derivative.
3. A method of claim 2 wherein the pegylated
derivative is N-terminally pegylated.
- 25 4. A method of claim 1 wherein said continuous
administration is accomplished by osmotic pump.
5. A DNA sequence according to SEQ ID No. 1.
- 30 6. A vector containing a DNA sequence
according to claim 5.
7. A vector of claim 6 wherein said vector is
pCFM1656.
- 35 8. A DNA sequence according to SEQ ID No. 3.

9. A vector containing a DNA sequence according to claim 8.

5 10. A vector according to claim 9 wherein said vector is pCFM1656.

10 11. A method of refolding partially purified OB protein in a solution obtained from inclusion bodies, said partially purified OB protein selected from the group consisting of:

 (a) recombinant methionyl murine OB protein (SEQ. ID. No. 2);

15 (b) recombinant methionyl human OB protein (SEQ ID No. 1);

 (c) the protein of (a) or (b) lacking the methionyl residue at position -1;

 wherein said refolding is accomplished using N-lauroyl sarcosine.

20

 12. A method of claim 11 wherein said sarcosine is used at a concentration of 0.5% - 2.0% weight per volume of solution.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Pelleymounter, Mary Ann
Hecht, Randy I
Mann, Michael B
- (ii) TITLE OF INVENTION: OB PROTEIN COMPOSITIONS AND METHODS
- (iii) NUMBER OF SEQUENCES: 6
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Amgen Inc.
(B) STREET: 1840 Dehavilland Drive
(C) CITY: Thousand Oaks
(D) STATE: California
(E) COUNTRY: U.S.A.
(F) ZIP: 91230-1789
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Pessin, Karol M.
(C) REFERENCE/DOCKET NUMBER: A-345A

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 491 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- | | |
|---|-----|
| TCTAGATTG AGTTTAACT TTTAGAAGGA GGAATAACAT ATGGTACCGA TCCAGAAAGT | 60 |
| TCAGGACGAC ACCAAAACCT TAATTAAAAC GATCGTTACG CGTATCAACG ACATCAGTCA | 120 |
| CACCCAGTCG GTCTCCGCTA AACAGCGTGT TACCGGTCTG GACTTCATCC CGGGTCTGCA | 180 |

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CCCGATCCTA AGCTTGTCCA AAATGGACCA GACCCTGGCT GTATACCAGC AGGTGTTAAC      240
CTCCCTGCCG TCCGAGAACG TTCTTCAGAT CGCTAACGAC CTCGAGAACC TTCGCGACCT      300
GCTGCACCTG CTGGCATTCT CCAAATCCTG CTCCCTGCCG CAGACCTCAG GTCTTCAGAA      360
ACCGGAATCC CTGGACGGGG TCCTGGAAGC ATCCCTGTAC AGCACCGAAG TTGTTGCTCT      420
GTCCCGTCTG CAGGGTTCCC TTCAGGACAT CCTTCAGCAG CTGGACGTTT CTCGGAATG      480
TTAATGGATC C                                         491

```

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 491 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

AGATCTAAAC TCAAAATTGA AAATCTTCCT CCTTATTGTA TACCATGGCT AGGTCTTTCA      60
AGTCCTGCTG TGGTTTTGGA ATTAATTTTG CTAGCAATGC GCATAGTTGC TGTAGTCAGT      120
GTGGGTCAGC CAGAGGCGAT TTGTCGCACA ATGGCCAGAC CTGAAGTAGG GCCCAGACGT      180
GGGCTAGGAT TCGAACAGGT TTTACCTGGT CTGGGACCGA CATATGGTCG TCCACAATTG      240
GAGGGACGGC AGGGTCTTGC AAGAAGTCTA GCGATTGCTG GAGCTCTTGG AAGCGCTGGA      300
CGACGTGGAC GACCGTAAGA GGTTTAGGAC GAGGGACGGC GTCTGGAGTC CAGAAGTCTT      360
TGGCCTTAGG GACCTGCCCC AGGACCTTCG TAGGGACATG TCGTGGCTTC AACAAACGAGA      420
CAGGGCAGAC GTCCCAAGGG AAGTCCTGTA GGAAGTCGTC GACCTGCAAA GAGGCCTTAC      480
AATTACCTAG G                                         491

```

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 147 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Met Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys
1 5 10 15

Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val Ser
20 25 30

Ala Lys Gln Arg Val Thr Gly Leu Asp Phe Ile Pro Gly Leu His Pro
35 40 45

Ile Leu Ser Leu Ser Lys Met Asp Gln Thr Leu Ala Val Tyr Gln Gln
50 55 60

Val Leu Thr Ser Leu Pro Ser Gln Asn Val Leu Gln Ile Ala Asn Asp
65 70 75 80

Leu Glu Asn Leu Arg Asp Leu Leu His Leu Leu Ala Phe Ser Lys Ser
85 90 95

Cys Ser Leu Pro Gln Thr Ser Gly Leu Gln Lys Pro Glu Ser Leu Asp
100 105 110

Gly Val Leu Glu Ala Ser Leu Tyr Ser Thr Glu Val Val Ala Leu Ser
115 120 125

Arg Leu Gln Gly Ser Leu Gln Asp Ile Leu Gln Gln Leu Asp Val Ser
130 135 140

Pro Glu Cys
145

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 454 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CATATGGTAC CGATCCAGAA AGTTCAGGAC GACACCAAAA CCTTAATTAA AACGATCGTT	60
ACGCGTATCA ACGACATCAG TCACACCCAG TCGGTGAGCT CTAAACAGCG TGTTACAGGC	120
CTGGACTTCA TCCCGGGTCT GCACCCGATC CTGACCTTGT CCAAAATGGA CCAGACCCTG	180
GCTGTATACC AGCAGATCTT AACCTCCATG CCGTCCCGTA ACGTTCTTCA GATCTCTAAC	240
GACCTCGAGA ACCTTCGCGA CCTGCTGCAC GTGCTGGCAT TCTCCAAATC CTGCCACCTG	300
CCATGGGCTT CAGGTCTTGA GACTCTGGAC TCTCTGGGCG GGGTCCTGGA AGCATCCGGT	360

TACAGCACCG AAGTTGTTGC TCTGTCCCGT CTGCAGGGTT CCCTTCAGGA CATGCTTTGG 420
CAGCTGGACC TGTCTCCGGG TTGTTAATGG ATCC 454

(2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 454 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTATACCATG GCTAGGTCTT TCAAGTCCGT CTGTGGTTTT GGAATTAATT TTGCTAGCAA 60
TGCGCATAGT TGCTGTAGTC AGTGTGGGTC AGCCACTCGA GATTTGTCGC ACAATGTCCG 120
GACCTGAAGT AGGGCCCAGA CGTGGGCTAG GACTGGAACA GGTTTTACCT GGTCTGGGAC 180
CGACATATGG TCGTCTAGAA TTGGAGGTAC GGCAGGGCAT TGCAAGAAGT CTAGAGATTG 240
CTGGAGCTCT TGGAAGCGCT GGACGACGTG CACGACCGTA AGAGGTTTAG GACGGTGGAC 300
GGTACCCGAA GTCCAGAACT CTGAGACCTG AGAGACCCGC CCCAGGACCT TCGTAGGCCA 360
ATGTCGTGGC TTCAACAACG AGACAGGGCA GACGTCCCAA GGGAAGTCCT GTACGAAACC 420
GTCGACCTGG ACAGAGGCCC AACAATTACC TAGG 454

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 147 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Val Pro Ile Gln Lys Val Gln Asp Asp Thr Lys Thr Leu Ile Lys
1 5 10 15
Thr Ile Val Thr Arg Ile Asn Asp Ile Ser His Thr Gln Ser Val Ser
20 25 30

Ser	Lys	Gln	Arg	Val	Thr	Gly	Leu	Asp	Phe	Ile	Pro	Gly	Leu	His	Pro
	35						40					45			
Ile	Leu	Thr	Leu	Ser	Lys	Met	Asp	Gln	Thr	Leu	Ala	Val	Tyr	Gln	Gln
	50					55					60				
Ile	Leu	Thr	Ser	Met	Pro	Ser	Arg	Asn	Val	Leu	Gln	Ile	Ser	Asn	Asp
65					70					75				80	
Leu	Glu	Asn	Leu	Arg	Asp	Leu	Leu	His	Val	Leu	Ala	Phe	Ser	Lys	Ser
				85					90					95	
Cys	His	Leu	Pro	Trp	Ala	Ser	Gly	Leu	Glu	Thr	Leu	Asp	Ser	Leu	Gly
			100					105					110		
Gly	Val	Leu	Glu	Ala	Ser	Gly	Tyr	Ser	Thr	Glu	Val	Val	Ala	Leu	Ser
		115					120					125			
Arg	Leu	Gln	Gly	Ser	Leu	Gln	Asp	Met	Leu	Trp	Gln	Leu	Asp	Leu	Ser
	130					135					140				
Pro	Gly	Cys													
145															

A B S T R A C T

The present invention provides methods and compositions for treating excess weight by administering
5 OB protein in a form for constant supply, at a dosage of less than or equal to about 1 mg protein/kg body weight/day. Compositions and methods used for production of recombinant murine and human OB protein are also provided. Compositions and methods for preparing
10 recombinant murine methionyl OB protein and recombinant human methionyl OB protein, including DNA sequences, vectors, host cells, methods of fermentation, and methods of purification are provided herein.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first, and sole inventor (if only one name is listed below) or a joint inventor (if plural names are listed below) of the invention entitled

OB PROTEIN COMPOSITIONS AND METHODS

which is described and claimed in the specification which:

☒ is attached hereto.
☐ was filed on _____
as Application Serial No.: _____
and was amended on _____ (if applicable)

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

Power of Attorney: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith:

Ron K. Levy, Registration No.: 31,539, Steven M. Odre, Registration No.: 29,094, and Karol M. Pessin, Registration No. 34,899, said attorney(s)/agent(s) to have in addition full power of revocation, including the power to revoke any power herein granted.

Please send all future correspondence to:

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Date: June 5, 1995

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Date of Deposit

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D. C. 20231

Paul F. Fehlner
Printed Name

Paul F. Fehlner 6/7/95
Signature

DECLARATION AND POWER OF ATTORNEY (cont'd)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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